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INITIAL ASSESSMENT OF HOST SUSCEPTIBILITY AND PATHOGEN VIRULENCE FOR CONSERVATION AND MANAGEMENT OF TASMANIAN AMPHIBIANS

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***Abstract.*—The disease chytridiomycosis, which is caused by lethal fungal pathogen *Batrachochytrium dendrobatidis*, is considered a threat to Tasmanian amphibians, but little is known about the susceptibility of Tasmania's amphibian species or the likely impacts of infections. We identified threatened and endemic species with prioritization rules and the aid of predictive risk models. We also conducted controlled infection experiments in order to test the pathogenicity of, and host susceptibility to, a Tasmanian isolate of *B. dendrobatidis*. Of the species prioritized for disease testing, the endemic Tasmanian Tree Frog (*Litoria burrowsae*) sustained high infection intensities and high (100%) mortality rates. The Green and Golden Frog (*Litoria raniformis*) became infected, but only 22% of exposed frogs died. Our results verify the pathogenicity of a local *B. dendrobatidis* strain and identify a highly vulnerable amphibian species, the Tasmanian Tree Frog. Our results are a critical component of Tasmanian conservation management programs, which are now enacting disease mitigation efforts. Thus, we demonstrate the importance of incorporating information on host susceptibility and *B. dendrobatidis* pathogenicity into risk analyses for management of amphibians threatened by chytridiomycosis.**

Key Words.—amphibian declines; *Batrachochytrium dendrobatidis;* chytridiomycosis; *Litoria burrowsae; Litoria raniformis;* species susceptibility; Tasmania

INTRODUCTION

Amphibians around the world are currently experiencing such severe population declines that they are considered to be the most threatened class of vertebrates (Stuart et al. 2004; Skerratt et al. 2007). Some of the most dramatic declines are attributed to the lethal disease chytridiomycosis, which is caused by the fungal pathogen *Batrachochytrium dendrobatidis* (hereafter "*Bd*"; Berger et al. 1998; Longcore et al. 1999). Initial outbreaks of chytridiomycosis are characterized by mass-mortality events that can occur in multiple species simultaneously (Lips et al. 2006; Woodhams et al. 2008). However, the effects of *Bd* infection differ among amphibian populations, communities, and species. In Australia, for example, some species have gone extinct (e.g., Sharp Snouted Day Frog, *Taudactylus acutirostris*; Schloegel et al. 2006), some remain severely depressed in the presence of endemic *Bd* infections (e.g., Armored Mist Frog; *Litoria lorica* [Puschendorf et al. 2011] and Southern Corroboree Frog, *Pseudophryne corroboree* [Hunter et al. 2010]), and some populations have persisted through initial outbreaks and seem to partially recover despite the ongoing presence of *Bd* (e.g., the Green-eyed Tree Frog, *Litoria genimaculata*; McDonald et al. 2005). Understanding the wide variation in the impacts of *Bd* remains a central challenge for conservation programs and wildlife management.

Similar to other serious emerging infectious diseases that cause population collapse (e.g., White-nose Syndrome in bats or Tasmanian Devil Facial Tumor Disease; Blehert et al. 2009; Jones et al. 2007), determining the most appropriate conservation strategies for chytridiomycosis is difficult, especially when multiple synergistic factors (e.g., host range restrictions, environmental degradation) may be at play. Among the more traditional options for disease management, such as disease suppression, vaccination and pathogen eradication, most are considered impractical on a large scale (e.g., eradication; DEH 2005) or are currently unavailable (e.g., vaccination; Stice & Briggs 2010) for combating chytridiomycosis in wild amphibians. Nonetheless, first-step management protocols have been suggested to mitigate the threat of chytridiomycosis for amphibian conservation (reviewed in Woodhams et al. 2011). These strategies include regional and global disease surveillance (Skerratt et al. 2008; World Organisation for Animal Health (OIE). 2008. Aquatic animal code. Available from http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/aquatic-animal-health-code/ [Accessed 7 February 2014]),

containing the spread of *Bd* (DEH 2005; Skerratt et al. 2007), establishing captive assurance programs (Mendelson et al. 2006), selecting for disease resistance or tolerance (Gascon et al. 2007), protecting natural disease refugia (Puschendorf et al. 2009, 2011), and promoting population recovery (DEH 2005) with the use of *in situ* treatment regimes (Voyles et al. 2012) or anti-fungal agents in the field (Harris et al. 2009).

The successful implementation of many of these management approaches will hinge on our ability to identify amphibian species threatened by chytridiomycosis that are in the greatest need of conservation intervention. It is also critical to identify lower risk species that may be tolerant of infection because they could transmit *Bd* to more susceptible species (Daszak et al. 2004). This problem requires information from numerous data domains, including information on the distribution and pathogenicity of *Bd* strains and the distribution, ecology, life-history, and other traits of amphibian hosts that mediate their susceptibility to infection and mortality at the individual and population levels (Murray et al. 2011a). Significant progress has been made using mathematical models. Several studies have used species distribution models to help inform disease risk assessments by identifying geographic regions that are likely to be environmentally suitable for the persistence of *Bd* (Ron 2005; Puschendorf et al. 2009; Rödder et al. 2009; Murray et al. 2011b), the life-history traits that correlate with species' declines (Lips et al. 2003), and ecological traits in *Bd*-positive host species (Bielby et al. 2008). More recently, studies have focused on integrating information on pathogen distribution/environmental requirements with host life-history and ecology for predicting species at risk of *Bd* infection (Murray and Skerratt 2012) or decline amidst multiple threats including chytridiomycosis (Murray et al. 2011a). These analyses have resulted in more accurate risk assessments for conservation management.

Although modeling approaches may help to identify geographical regions and/or particular species (or groups of species) at risk of infection or decline, their use has some important limitations. First and foremost, they are correlative and often based on incomplete information or data with sampling biases. Thus, they may have limited capacity to predict idiosyncratic factors such as relative virulence of particular *Bd* strains, a host species' inherent immunity to infection, or disease development. A critical step that will achieve a more accurate risk assessment for any particular species, will be the explicit testing of host susceptibility to infection and the pathogenicity of local *Bd* strains (Blaustein et al. 2005; Bancroft et al. 2011; Gahl et al. 2011; Searle et al. 2011; Gervasi et al. 2013; Olson et al. 2013).

The herpetofauna of Tasmania provides an excellent model system to apply these principles and establish an informed management scheme for chytridiomycosis. Tasmania is predicted to be highly suitable for the persistence of *Bd* within Australia, comparable to many parts of the eastern and southern seaboards where declines and extinctions have occurred (Murray et al. 2011b). The Tasmanian Wilderness World Heritage Area (TWWHA), which provides core habitat for the three endemic species (Driessen and Mallick 2003), appeared to be largely free of *Bd* in a preliminary survey (Pauza et al. 2010). Models predict that *Bd* has simply not yet dispersed to these regions (Murray et al. 2011b) and that species in this region are at risk of decline (Murray et al. 2011a). These findings suggest that there is an opportunity to establish management priorities and implement conservation efforts to abate the threat of severe decline in Tasmanian amphibians, especially in the TWWHA.

The objectives of our study were to identify target species of concern using predictive models and any evidence of previous decline or susceptibility in other areas of Australia, test the pathogenicity of a *Bd* isolate endemic to the region, and determine the relative innate susceptibility and disease development characteristics of Tasmanian species at risk. In meeting these objectives we make improved recommendations for the management of Tasmanian amphibian species.

MATERIALS AND METHODS

There are 11 species of amphibians identified in Tasmania (Littlejohn 2003). Unfortunately, testing all 11 species was not possible for practical and ethical reasons. We therefore identified key target species for an intensive investigation on chytridiomycosis. In order to develop a species "short list" for our investigation, we integrated information from multiple sources: 1) species endemism and current conservation status, 2) predictive models of risk of pathogen exposure and host decline, and 3) previous studies on host susceptibility and anecdotal evidence of species declines, both in Tasmania and on mainland Australia.

Species endemism and conservation status are exceptionally important criteria for conservation prioritization (Skerratt et al. 2008). Therefore, we first prioritized species by whether they are endemic to Tasmania, such as the Tasmanian Tree Frog (*Litoria burrowsae*). We also prioritized species that are listed as vulnerable/endangered, which included the Green and Golden frog (*Litoria raniformis*; Tasmanian Threatened Species Protection Act 1995; Australian Environmental Protection and Biodiversity Conservation Act 1999; International Union for Conservation of Nature. 2009. The IUCN Red List of Threatened Species. Available from: www.iucnredlist.org [Accessed 7 February 2014]. Mortality associated with chytridiomycosis has been observed in this species (Waldman et al. 2001; Berger et al. 2004), but some infection trials (Carver et al.

2010) suggest it is tolerant. Thus, the relative susceptibility of *L. raniformis* to chytridiomycosis is unclear.

Second, we used predictive models to help identify other important species based on potential host exposure (i.e., an overlap in predicted environmental suitability for *Bd* and host species distributions), and host life-history and ecological traits (Murray et al. 2011a; Murray et al. 2011b; Murray and Skerratt 2012). These included the Smooth Froglet (*Geocrinia laevis*) and the Southern Toadlet (*Pseudophryne semimarmorata*). Unfortunately, these species, along with two of the endemic species (*Crinia tasmaniensis* and *Bryobatrachus nimbus*) were not collected in sufficient numbers for testing.

Third, we conducted a literature review for information on species susceptibility to chytridiomycosis. For example, susceptibility trials were already conducted on Spotted Marsh Frogs (*Limnodynastes tasmaniensis*) and Striped Marsh Frogs (*Limnodynastes peronii*). These species were relatively unaffected in previous exposure experiments (Woodhams et al. 2007; Stockwell et al. 2010). Additionally, field studies indicate that the Brown Tree Frog (*Litoria ewingii*), the Banjo Frog (*Limnodynastes dumerilii*), and Common Froglet (*Crinia signifera*) are still widespread and highly abundant despite *Bd* infection (Obendorf and Dalton 2006). Additionally, predictive models suggested that these species are unlikely to be at risk of decline from *Bd* (Murray et al. 2011a).

Obtaining a local Bd isolate.—We isolated a new strain of *Bd* from a diseased *Limnodynastes peronii* tadpole found at Couta Rocks, Tasmania. We named the new *Bd* isolate CoutaRocks-L.peronii-09-LB-P3 as per standardized format (Berger et al. 2005). The range of *L. peronii* overlaps with both *L. raniformis* and *L. burrowsae* in the Northwest corner of Tasmania (Fig. 1), and therefore made this an ideal local isolate for testing *Bd* virulence. This isolate was purified and cultured on tryptone/gelatin hydrolysate/lactose (TGhL) agar with antibiotics, and then passaged into liquid TGhL broth (Longcore et al. 1999) in 25-cm^2 cell culture flasks. Cultures were maintained at James Cook University in TGhL broth at 4° C until they were transported to Newtown Laboratories, of the Department of Primary Industries, Parks, Water and the Environment (DPIPWE), Tasmania, Australia, where they were held at 23° C and passaged every 4–7 days.

Experimental inoculations.—We collected three amphibian species for exposure experiments: the two Tasmanian species described above (*L. raniformis* and *L. burrowsae*) and one species that is not found in Tasmania, but is known to be highly susceptible to chytridiomycosis, the Common Green Tree Frog (*Litoria caerulea*; Berger et al. 2005; Woodhams et al. 2007; Voyles et al. 2009), which was collected in Queensland and used as a positive control. Frog collections occurred in January and February 2009. We collected each animal by hand using clean vinyl gloves, transferred it to an individual plastic container (200 × 240 × 330 mm), and transported the animal to temperature (18−23º C) and light (12 h light/12 h dark) controlled facilities. We collected adult *L. raniformis* (n = 19, mean mass: 28.67 g ± 6.65 SD) from Low Head in northeast Tasmania. We collected adult *L. burrowsae* (n = 6, mean mass: 19.25 g ± 8.10 SD) near Melaleuca in the TWWHA. We collected adult *L. caerulea* (n = 19, mean mass: 43.71 g ± 7.70 SD) from residential areas of Townsville, Queensland.

We followed protocols outlined for other exposure experiments (Berger et al. 2005; Voyles et al. 2009). We fed frogs vitamin-dusted crickets (medium-sized, Pisces Enterprises, Kenmore, Australia) *ad libitum* and changed tap water (250 mL) twice per week. We collected a skin swab sample when the frogs arrived at the laboratory to confirm frogs were not infected with *Bd* before inoculations. Collecting skin swab samples is a non-invasive technique that involves rubbing a sterile cotton swab (Medical Wire & Equipment, Corsham, UK) over the ventral surfaces and digits (Hyatt et al. 2007). We performed polymerase chain reaction (PCR) for *Bd* using standard protocols (Taqman real-time PCR assay, Boyle et al. 2004), with standards provided by Alex Hyatt (Australian Animal Health Laboratory, Geelong, Australia).

We randomly assigned frogs to exposure and control groups. We harvested zoospores by filtering liquid cultures through sterile filter paper to remove sporangia. We determined zoospore concentrations using a haemocytometer (Improved Neubauer Bright-line, Hausser Scientific, Pennsylvania, USA) and adjusted the concentration as needed by addition of dilute salt solution (in mMol: 1.0 KH_2PO_4, 0.2 $CaCl_2$ H_2O, 0.1 $MgCl_2$ $2H_2O$). We exposed frogs to *Bd* zoospores in plastic containers via shallow immersion in a bath of the exposure solution. We held frogs in exposure groups in round plastic containers (12 cm diameter × 6.5 cm tall) in a bath solution (20% Holtfretter's solution: 250 mL, in mMol: 6.0 NaCl, 0.06 KCL, 0.09 CaCl2, 0.24 NaCO3; pH 6.5) inoculated with 1.5×10^6 *Bd* zoospores. We held uninfected control frogs in a bath of Holtfretter's solution with equal quantities of added dilute salt solution collected from sterile TGhL without *Bd*. After 24 h we moved frogs to fresh containers with 20% Holtfretter's solution (250 mL), which we replaced with tap water (250 mL) after three days. We monitored the frogs daily for changes in behavior and clinical signs of chytridiomycosis (as described in Voyles et al. 2009).

We used a Mantel-Cox log rank test for censored survival data (SPSS Statistics 17.0, SPSS Inc Illinois, USA) to analyze survival curves in exposure experiments. To evaluate other parameters such as mass, body condition, and intensity of infection, we first tested for normality (Shapiro-Wilk's test) and

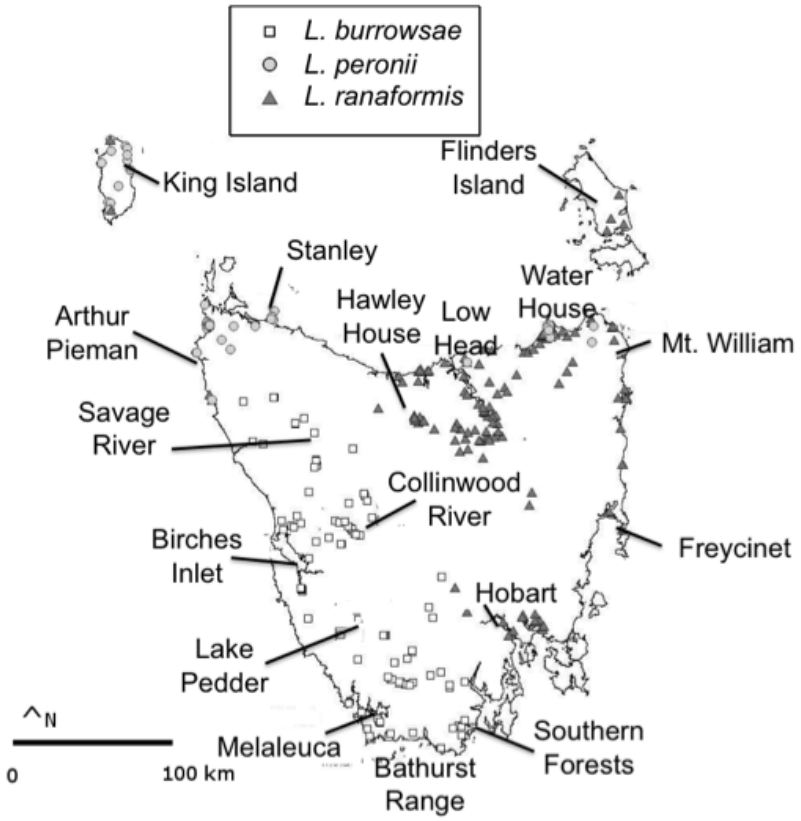

FIGURE 1. Historical locations of three Tasmanian amphibian species compiled from the Natural Values Atlas of Tasmania (www.naturalvaluesatlas.tas.gov.au) [Accessed February 7, 2014] for populations of *Limnodynastes peronii* populations (grey circles), *Litoria raniformis* (dark triangles), and *Litoria burrowsae* populations (open squares). We collected the isolate of *Batrachochytrium dendrobatidis* (*Bd*) used in this study from a diseased *L. peronii* tadpole found in the Arthur Pieman Conservation area. The range of *L. peronii* overlaps with both *L. raniformis* and *L. burrowsae* in the Northwest corner of Tasmania.

homogeneity of variance (Levene's test).

We used nonparametric tests (Mann Whitney and Kruskal-Wallis tests) when the assumptions of parametric tests were violated and could not be corrected by transformation. We log-transformed the results for PCR prior to statistical testing. We considered results statistically significant at $P < 0.05$.

RESULTS

We confirmed that the frog species that were used in our infection experiment (*Litoria burrowsae, Litoria raniformis*, and *Litoria caerulea*) were negative for *Bd* with PCR analysis before exposures. Additionally, none of the frogs exhibited any clinical signs of chytridiomycosis and there were no significant differences in mass or body condition among treatment groups. All the *L. caerulea* (9/9) and *L. burrowsae* (3/3) control (unexposed) frogs survived to the termination of the experiment at day 140. One *L. raniformis* control frog died early in the experiment, at day 24. This frog tested negative for *Bd* infection throughout the experiment and the cause of death in this individual is unknown.

The *Bd* isolate that we obtained from a diseased *L. peronii* tadpole (isolate CoutaRocks-L.peronii-09-LB) caused 100% mortality in the exposed *L. burrowsae* group (mean infection intensity 6,294 ± S.D. 9,023; n = 3; Fig. 2) and in our positive control species, *L. caerulea* (mean infection intensity 15,001 ± S.D. 5,267; n = 10; Fig. 2). However, only 2/9 (22%) of the exposed *L. raniformis* frogs died, with most surviving to the termination of the experiment (mean infection intensity 240.56 ± S.D. 717.17; n = 9; Fig. 2). Prior to mortality, we observed classical clinical signs of chytridiomycosis including lethargy, inappetence, cutaneous erythema, irregular skin sloughing, abnormal posture (hind legs abducted), and loss of righting reflex (as described in Voyles et al. 2009) in the exposed *L. burrowsae* group and the exposed *L. caerulea* group. However, we did not observe these clinical signs of chytridiomycosis in the exposed *L. raniformis* group.

Although all exposed *L. burrowsae* and *L. caerulea* died, the time until death differed significantly between these two species (log rank test on censored survival data, $P < 0.001$; Fig. 2A). Survival in both of these species was significantly different between

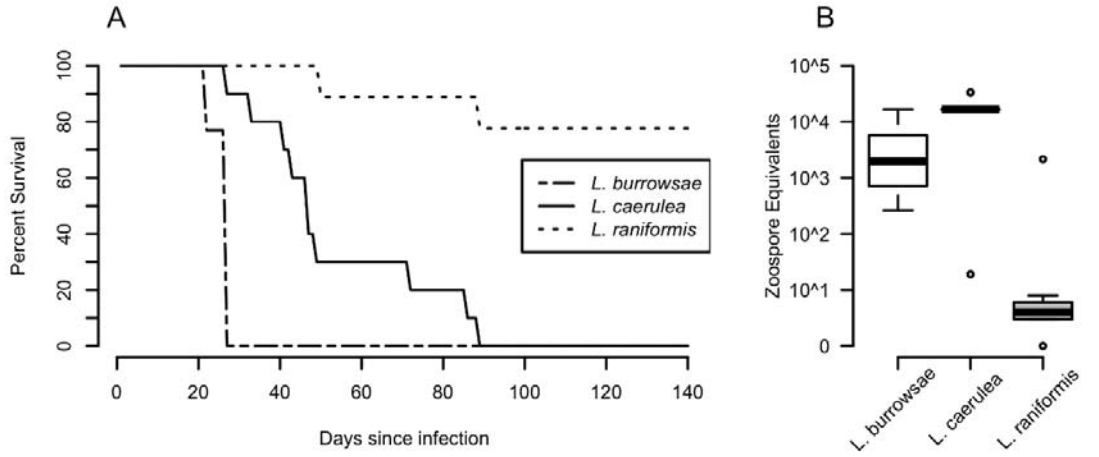

FIGURE 2. (A) Patterns of survival of *Litoria burrowsae* (n = 3), *Litoria caerulea* (n = 10) and *Litoria raniformis* (n = 9) that were experimentally exposed to *Batrachochytrium dendrobatidis* (isolate CoutraRocks-L.peronii-09-LB-P3, obtained from Tasmania). (B) Box plot with median infection intensities (dark horizontal bands) with first and third quartiles (box limits), range (vertical lines), and outliers (open circles) in experimentally infected *L. burrowsae* (n = 3), *L. caerulea* (n = 10), and *L. raniformis* (n = 9). Zoospore equivalents were determined using polymerase chain reaction (PCR) on skin swab samples collected at the time of death or at the termination of the experiment at day 140.

control and exposure groups (log rank test on censored survival data, *L. burrowsae*, $P = 0.03$; *L. caerulea*, $P < 0.001$; Fig. 2A). In contrast, survival of *L. raniformis* was not significantly different between control and exposure groups (log rank test on censored survival data, $P = 0.281$).

Infection intensities were measured in groups exposed to *Bd* at death or at the termination of the experiment for those frogs that survived. Zoospore equivalents (*Bd*-loads) were significantly different among species (Kruskal-Wallis, $P < 0.001$). The *L. raniformis* group had significantly lower *Bd*-loads than the *L. caerulea* group (log rank test, $P < 0.001$) and the *L. burrowsae* group (log rank test, $P = 0.03$; Fig. 2B).

DISCUSSION

Our study demonstrates differential susceptibility to chytridiomycosis in two Tasmanian amphibian species. In our trials, all endemic *L. burrowsae* frogs that were exposed to the pathogen became infected and died with chytridiomycosis. In contrast, endangered *Litoria raniformis* frogs became infected with *Bd*, but only a small proportion of infected frogs developed fatal chytridiomycosis (Fig. 2A), indicating tolerance to *Bd* infection. Our study therefore provides a best initial estimate of the threat of chytridiomycosis for two species of conservation concern and now serves as the basis of the Tasmanian Chytrid Management Plan, which provides a full risk assessment for Tasmanian amphibians (Philips et al. 2010). Accordingly, Tasmanian management agencies are making crucial first steps in amphibian conservation efforts: initiating disease mitigation practices for populations of *L. burrowsae* and directing resources to investigate other threats to *L. raniformis*.

Our susceptibility trials demonstrate that *L. burrowsae* frogs are probably highly susceptible to chytridiomycosis. Although we had a relatively small sample size of our experimental group (n = 3), the high rate of mortality (100%) is concerning because new evidence suggests that *Bd* may be spreading into *L. burrowsae* populations in Western Tasmania (Philips et al. 2010). Two previous field studies suggested that the Tasmanian Wilderness World Heritage Area (TWWHA), which incorporates most of the distribution of *L. burrowsae*, is largely free of *Bd* (Pauza et al. 2010). However, *Bd* was more recently recorded at four locations within the TWWHA boundary. Therefore, we suggest that the *L. burrowsae* populations along the eastern edge of the TWWHA, especially those populations near areas where *Bd* has been detected, should be a top priority for continued monitoring, focused investigations on chytridiomycosis, and developing contingency plans to respond to chytridiomycosis outbreaks. Because any effective emergency conservation measure is likely to be costly and difficult to implement, the management of declining species should be outlined *a priori*. For example, additional conservation actions for naïve *L. burrowsae* populations should include establishing captive assurance colonies and optimizing species-specific antifungal treatments (Berger et al. 2010; Philips et al. 2010). Such tools can be used in long-term survival assurance captive management, in short-term *ex situ* husbandry programs, or for *in situ* treatment regimes in the event of an outbreak of chytridiomycosis.

The overall pattern of survival in our study indicates that *L. raniformis* frogs, at least those from populations in northern Tasmania, do not appear to be highly susceptible to chytridiomycosis. As noted above,

results from other *L. raniformis* investigations have produced mixed results. Chytridiomycosis-associated mortality has been observed in the wild (Waldman et al. 2001; Berger et al. 2004) yet in inoculation trials in New Zealand, *L. raniformis* cleared *Bd* infection and survived (Carver et al. 2010). Murray et al. (2011a) also suggested an equivocal role of *Bd* in this species' decline based on model predictions. In Tasmania, tolerance may have evolved in populations that survived initial declines, or they may be an inherently less susceptible population of *L. raniformis*. In a study of life-history traits of *L. raniformis* and other Australian frog species, Heard et al. (2012) suggested that metapopulation dynamics of *L. raniformis* make this species disproportionately vulnerable to habitat loss, environmental degradation, and fragmentation. These mechanisms have yet to be investigated in Tasmania. We propose that evaluating other threatening processes, such as habitat loss and degradation, should be a high priority for this species throughout their historic range in Tasmania. Also, we suggest screening of *Bd* should continue because amphibians with high infection tolerance can act as disease reservoirs and lead to *Bd* transmission to other native species that are more susceptible to disease (Daszak et al. 2004; Woodhams et al. 2008).

In addition to further investigations on chytridiomycosis for *L. burrowsae*, and screening for *Bd* in *L. raniformis*, we suggest that research on chytridiomycosis continue for other Tasmanian species. We have limited information for *Geocrinia laevis*, *Pseudophryne semimarmorata*, *Crinia tasmaniensis*, and *Bryobatrachus nimbus* and research on these species should be prioritized. *Bryobatrachus nimbus* is an endemic species with a small montane range. Thus, determining its susceptibility will be important for Tasmanian conservation organizations, especially in light of model predictions (Murray et al. 2011a, 2011b; Murray and Skerratt 2012), which suggest this species could be both a host for *Bd* and a possible declining species. Many *B. nimbus* populations are found in the same geographical regions as *L. burrowsae* and could therefore be incorporated into intervention programs for chytridiomycosis, creating a more holistic management approach for all Tasmanian amphibians.

Our study offers an approach to apply the best available knowledge on the disease ecology of chytridiomycosis to estimate disease risk, and to direct more informed research and management schemes. Because our results have helped develop the Tasmanian Chytrid Management Plan, which provides a management strategy for Tasmanian amphibians (Philips et al. 2010), additional research, monitoring, and disease mitigation efforts are currently underway in Tasmania. For example, a program for *ex situ* management of *L. burrowsae* has been initiated and screening for *Bd*-infection is continuing with support from the State of Tasmania, Department of Primary Industries, Parks, Water and the Environment, and Natural Resource Management South. This combined effort by management agencies, as directed by results from our study, represents a significant step forward for amphibian conservation in Tasmania. Additionally, our study (and other species susceptibility studies) will be important for improving predictive models for understanding chytridiomycosis. Thus, we provide an example approach for amphibian researchers and wildlife managers to come together and address a seemingly intractable conservation problem.

The global loss of amphibian biodiversity has been called "the greatest species conservation challenge in the history of humanity" (Zippel and Mendelson 2008) and chytridiomycosis is an important contributing factor. Although habitat degradation and destruction may also be contributing to population declines and loss in Tasmania, we focused on assessing the threat of chytridiomycosis for Tasmanian amphibians. With this method of assessing the risk of severe disease, Tasmanian conservation programs are now better prepared for any additional outbreaks of chytridiomycosis in naïve populations and may be able to facilitate recovery in already infected populations. Our work can serve as a model approach for guiding amphibian conservation in management organizations around the world.

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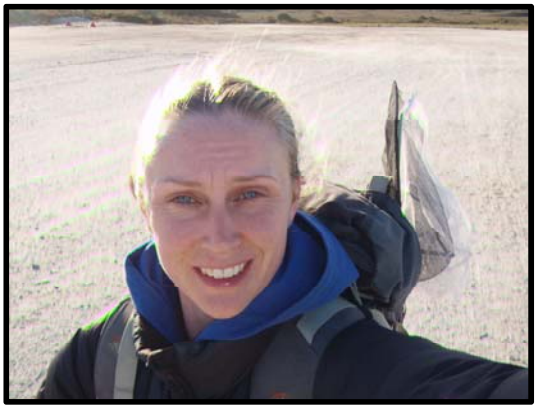

JAMIE VOYLES has been studying chytridiomycosis in amphibians since 2001. She received a Bachelor of Arts degree from the University of Washington in Seattle, Washington and a Masters of Science degree from the University of Colorado in Boulder, Colorado. Her doctoral work was conducted at James Cook University in Townsville, Australia and focused on amphibian chytridiomycosis. Following the completion of her doctoral work, she worked for the State of Tasmania, Biodiversity Conservation Branch, to help develop a disease oriented management plan for Tasmanian amphibians. She continued her research in postdoctoral positions at the University of Idaho and the University of California, Berkeley. She is currently an Assistant Professor at New Mexico Tech and conducts chytridiomycosis research in Central America and in California. She is a member of multiple working groups investigating disease-related amphibian declines: Integrated Research Challenges in Environmental Biology (IRCEB), Amphibian Disease Ecology Group (ADEG), Amphibian Survival Alliance, and the One Health Research Group. She is actively involved in conservation initiatives, such as Amphibian Rescue and Conservation Project, and contributes to amphibianrescue.org and AmphibiaWeb. (Photographed by Jamie Voyles).

ANNIE PHILIPS graduated from Sydney University with a Bachelor's degree in Veterinary Science in 1988. She worked as a clinical veterinarian in a variety of clinics worldwide with a variety of domestic and wildlife species until obtaining a Master degree in Tropical Environmental Management in 2001. Various positions over this period included wildlife disease research in the Galapagos Islands and Antarctica, dog health programs in the Indian Himalayas and Arnhem Land, and working in Shanghai helping establish the first western veterinary clinic in mainland China. Annie currently works as a wildlife veterinarian for the Department of Primary Industries, Parks, Water and Environment in Tasmania. She manages the Tasmanian Chytrid Research and Management Program, works on biosecurity, disease surveillance, wildlife management in emergencies, and a number of other issues. She is on the Antarctic Animal Ethics Committee and is the Tasmanian State Coordinator for the Australian Wildlife Health Network. (Photographed by Barry Baker).

MICHAEL DREIESSEN has worked as a zoologist for the Tasmanian Government for over 25 years in the area of wildlife conservation and management, particularly in the Tasmanian Wilderness World Heritage Area (TWWHA). It is through this work that Michael became aware of the threat of chytridiomycosis on three frog species that are largely restricted to the TWWHA and initiated a program to investigate and manage the impact of this disease. In addition to disease, Michael has undertaken research to improve understanding and management of fire, introduced animals, hunting, and climate change on a diverse range of animals including wallabies, bandicoots, bettongs, platypus, glow-worms, and pygmy shrimps. Michael has published over 40 peer-reviewed papers. The role of fire in wildlife conservation has been a key area of management and research focus and he is currently undertaking a Ph.D. at the University of Tasmania investigating invertebrate succession following low intensity burns in buttongrass moorlands. (Photographed by Barry Baker).

MATTHEW WEBB works on biodiversity research and conservation management and has focused on gaining a better understanding the ecological requirements and threats faced by endangered species. He is currently employed as a Threatened Species Zoologist in the Biodiversity Conservation Branch with the Tasmanian Government where he has worked on a range of threatened taxa (frogs, skinks, freshwater crayfish, marine mammals, and several bird species), and topics including landscape-scale conservation management, impacts of native forest logging, disease surveillance and management, and other biodiversity monitoring programs. (Photographed by Mark Holdsworth).

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DOUGLAS C. WOODHAMS has been studying disease ecology in amphibians since 1998. He received a Bachelor of Science from Michigan State University and a Ph.D. from James Cook University followed by research associate experience at Vanderbilt University, James Madison University, and the University of Zurich. Currently in the Department of Ecology and Evolutionary Biology at the University of Colorado, Boulder, his research focuses on host-symbiont-pathogen biology and immunological ecology. To date, Dr. Woodhams has 38 peer-reviewed publications focused on all aspects of amphibian chytridiomycosis. Current research surrounds the hypothesis that microbiota extend host immune defenses and can be altered through probiotic therapy to increase disease resistance in amphibians and reduce vector competence of mosquitoes. (Photographed by Tim Carr).

KRIS MURRAY received his Ph.D. from the University of Queensland, Australia in 2010, after working on the impacts and dynamics of amphibian chytridiomycosis. After finishing his Ph.D., he took up a post-doctoral position with the School of Public Health and Tropical Medicine at James Cook University in Queensland, Australia. In this position, Kris focused on evaluating the utility of the multidisciplinary 'One Health' paradigm as a model for collectively coordinating and improving human, animal, and environmental health. He is currently a research scientist at EcoHealth Alliance, as an ecologist and conservation biologist with broad interests in biodiversity, evolution, behavior, and disease ecology. Kris currently investigates the roles of biodiversity, land- use change, climate change, and other socio-economic, demographic, and environmental drivers in disease emergence and conservation. (Photographed by Domanique Murray).

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